AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the applications:

Listing of Claims:

Claims 1-52 (canceled)

53. (currently amended) A method for assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity in a cell free or cell culture system comprising the steps of:

(a) contacting hu-Asp1 enzyme with an amyloid precursor protein (APP) substrate, wherein the hu-Asp1 enzyme is a recombinant polypeptide expressed by a host cell transformed or transfected with a nucleic acid molecule that comprises a nucleotide sequence that encodes an amino acid sequence at least 95% identical to SEQ ID NO: 2 or to a fragment of SEQ ID NO: 2 ~~that retains α-secretase activity~~, wherein the polypeptide retains ~~α-secretase~~ APP proteolytic activity, and wherein said substrate contains ~~an~~ α-secretase a hu-Asp1 APP cleavage site; and

(b) measuring cleavage of the APP substrate at the ~~α-~~ hu-Asp1 APP cleavage site, thereby assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity.

54. (canceled)

55. (currently amended) A method for assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity in a cell free or cell culture system comprising the steps of:

(a) contacting a hu-Asp1 enzyme with an amyloid precursor protein (APP) substrate, wherein said substrate contains an ~~α-secretase~~ a hu-Asp1 APP cleavage site, wherein the hu-Asp1 enzyme is a purified and isolated polypeptide comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 2 or to a fragment of SEQ ID NO: 2 ~~that retains α-secretase activity~~, wherein the polypeptide retains ~~α-secretase~~ APP proteolytic activity; and

(b) measuring cleavage of the APP substrate at the α- hu-Asp1 APP cleavage site, thereby assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity.

56. (currently amended) ~~A~~ The method according to any one of claims 53, 55, 79, or 80 wherein the polypeptide lacks a transmembrane domain.

57. (currently amended) ~~A~~ The method according to claim 78, wherein the polypeptide lacks transmembrane amino acids 469-492 of SEQ ID NO: 2.

58. (currently amended) ~~A~~ The method according to claim 57, wherein the polypeptide further lacks the cytoplasmic domain amino acids 493-518 of SEQ ID NO: 2.

59. (currently amended) ~~A~~ The method according to claim 57, wherein the polypeptide further lacks amino terminal amino acids 1-62 of SEQ ID NO: 2.

60. (currently amended) A The method according to claim 53 or 79, wherein the contacting step comprises growing the host cell under conditions in which the cell expresses the hu-Asp1 enzyme in the presence of the APP substrate.

61. (currently amended) ~~A~~ The method of claim 60, wherein said cell further expresses a polynucleotide encoding an APP substrate containing ~~an α-secretase~~ a hu-Asp1 cleavage site, and wherein the contacting step further comprises growing the cell under conditions in which the cell expresses the hu-Asp1 enzyme and the APP substrate.

62. (currently amended) ~~A~~ The method according to any one of claims 53, 55, 79, ~~and~~ or 80 wherein the APP substrate hu-Asp1 cleavage site comprises the amino acid sequence LVFFAEDF (SEQ ID NO: 84) or KLVFFAED (SEQ ID NO: 73).

63. (currently amended) ~~A~~ The method of claim 62, wherein the APP substrate comprises a detectable label.

64. (currently amended) ~~A~~ The method of claim 63, wherein the detectable label is selected from the group consisting of radioactive labels, enzymatic labels, and fluorescent labels.

65. (currently amended) ~~A~~ The method according to any one of claims 53, 55, 79, and or 80 wherein the APP substrate comprises a human APP isoform and further comprises a carboxy-terminal di-lysine.

66. (currently amended) ~~A~~ The method according to any one of claims 53, 55, 79 ~~and~~ or 80, wherein the APP substrate comprises a human APP isoform and the determining step comprises measuring the production of amyloid alpha peptide (sAPPα).

Claims 67-77 (canceled)

78. (currently amended) ~~A~~ The method according to any one of claims 53, 55, 79, ~~and~~ or 80, wherein the the polypeptide comprises amino acids 63-468 of SEQ ID NO: 2.

79. (currently amended) A method for assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity in a cell free or cell culture system comprising the steps of:

(a) contacting a hu-Asp1 enzyme with an amyloid precursor protein (APP) substrate, wherein said substrate contains ~~an α-secretase~~ a hu-Asp1 APP cleavage site, wherein the hu-Asp1 enzyme is a recombinant polypeptide having ~~α-secretase~~ APP proteolytic activity, and wherein said polypeptide is expressed by a host cell transformed or tranfected with a nucleotide sequence that encodes the polypeptide and hybridizes under the following stringent conditions to the complement of SEQ ID NO: 1:

(1) hybridization at 42°C in a hybridization buffer comprising 6x SSC and 0.1% SDS, and

(2) washing at 65°C in a wash solution comprising 1x SSC and 0.1% SDS; and

(b) measuring cleavage of the APP substrate at the α- hu-Asp1 APP cleavage site, thereby assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity.

80. (currently amended) A method for assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity in a cell free or cell culture system comprising the steps of:

(a) contacting a hu-Asp1 enzyme with an amyloid precursor protein (APP) substrate, wherein the substrate contains ~~an α-secretase~~ a hu-Asp1 APP cleavage site, wherein the hu-Asp1 enzyme is a purified and isolated polypeptide comprising an amino acid sequence encoded by a nucleotide sequence that hybridizes under the following stringent conditions to the complement of SEQ ID NO: 1:

(1) hybridization at 42°C in a hybridization buffer comprising 6x SSC and 0.1% SDS, and

(2) washing at 65°C in a wash solution comprising 1x SSC and 0.1% SDS; and

(b) measuring cleavage of the APP substrate at the ~~α-~~ hu-Asp1 APP cleavage site, thereby assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity.

81. (currently amended) A method for assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity in a cell free or cell culture system comprising the steps of:

(a) contacting a hu-Asp1 enzyme with a amyloid precursor protein (APP) substrate, wherein the hu-Asp1 enzyme is a polypeptide with α-secretase activity, wherein the polypeptide comprises an amino acid sequence at least 95% identical to SEQ ID NO: 2 or to a fragment of SEQ ID NO: 2 that retains ~~α-secretase~~ APP proteolytic activity, wherein said substrate is a human APP isoform comprising an ~~α-secretase~~ hu-Asp1 APP cleavage site and a carboxy di-lysine; and

(b) measuring cleavage of the APP substrate at the ~~α-~~ hu-Asp1 APP cleavage site, thereby assaying hu-Asp1 ~~α-secretase~~ APP proteolytic activity.

82. (new) The method according to any one of claims 53, 55, 79, or 80 wherein the APP substrate hu-Asp1 APP cleavage site comprises the amino acid sequence EVKMDAEF (SEQ ID NO: 70) or EVNLDAEF (SEQ ID NO: 71).

83. (new) A method of modulating the enzymatic production of β-amyloid peptide (Aβ) from β-amyloid precursor protein (APP) or a fragment thereof, comprising contacting said APP or APP fragment with a BACE2 polypeptide or an agonist or antagonist thereof.

84. (new) The method of claim 83 wherein said APP is a native sequence human APP.

85. (new) The method of claim 83 wherein said APP is the 695-amino acid isotype.

86. (new) The method of claim 83 wherein said APP contains the Swedish mutation.

87. (new) The method of claim 83 wherein said APP fragment is β-CTF.

88. (new) The method of claim 83 wherein said BACE2 is a native sequence BACE2 polypeptide.

89. (new) A method of inhibiting the formation of a β-amyloid peptide (Aβ) from β-amyloid precursor protein (APP) or a fragment thereof, comprising contacting said APP or APP fragment with a BACE2 polypeptide or an agonist thereof.

90. (new) The method of claim 89 wherein said APP is a native sequence human APP.

91. (new) The method of claim 89 wherein said APP is the 695-amino acid isotype.

92. (new) The method of claim 90 wherein said APP contains the Swedish mutation.

93. (new) The method of claim 89 wherein said APP fragment is β-CTF.

94. (new) The method of claim 89 wherein said BACE2 is a native sequence BACE2 polypeptide.

95. (new) The method of claim 89 which is performed in the presence of an α-secretase activity.

96. (new) The method of claim 89 which is performed in the presence of a γ-secretase activity.

97. (new) The method of claim 89 which is performed in the presence of a β-secretase activity other than BACE2.

98. (new) The method of claim 97 wherein said β-secretase activity in due to the presence of an enzyme having a pH optimum at about pH 6.5-7.0, and an estimated molecular weight of about 32-39 kDa as calculated from radiation inactivation analysis of HEK293 cell membrane extracts, or about 20-26 kDa as calculated from radiation inactivation analysis of human brain samples, with a candidate compound.

99. (new) The method of claim 97 wherein said β-secretase activity is due to the presence of a β-secretase enzyme having a pH optimum at about pH 4.5-5.0 and an estimated molecular weight of about 50-60 kDa as calculated from radiation inactivation analysis of HEK293 cell membrane extracts or human brain samples (BACE1).

100. (new) The method of claim 89 wherein said BACE2 is in isolated form.

101. (new) The method of claim 89 wherein said BACE2 is in immobilized or cell bound form.

102. (new) The method of claim 89 wherein the APP or APP fragment is contacted with an agonist of BACE2.

103. (new) The method of claim 102 wherein said agonist stimulates the production of BACE2.

104. (new) The method of claim 102 wherein said agonist enhances the activity of BACE2.

105. (new) The method of claim 102 wherein said agonist mimics the activity of BACE2.

106. (new) The method of claim 102 wherein said agonist is a small molecule.

107. (new) A method of inhibiting the release of a full-length β-amyloid (Aβ) polypeptide from ±3-amyloid precursor protein (APP) or a fragment thereof, comprising cleaving said APP or APP fragment by a BACE2 polypeptide or an agonist thereof at a site interfering with β-secretase processing of said APP or APP fragment.

108. (new) The method of claim 107 wherein said site is at or around the α-secretase cleavage site of native sequence APP or a fragment thereof.

109. (new) The method of claim 108 wherein said site is within about 10 amino acids on either side of said β-secretase cleavage site.

110. (new) A method of modulating APP processing activity comprising contacting APP with a modulator of Asp1 APP processing activity, thereby modulating the production of amyloid beta peptide.

111. (new) A method of claim 110, wherein modulation of production of amyloid beta is a treatment for Alzheimer's disease.

112. (new) A method of claim 110 further comprising increasing Asp1 induced cleavage between residues phe^{20} and ala^{21} of the amyloid beta peptide.

113. (new) A method of claim 110 further comprising increasing Asp1 induced cleavage between residues phe^{19} and phe^{20} of the amyloid beta peptide.

114. (new) A method of claim 110 further comprising inhibiting Asp1 induced cleavage between residues KMDA or NLDA of the amyloid beta peptide.